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A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi

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Abstract

Understanding the contributions of soil microorganisms to soil stabilization at the molecular level will lead to ways to enhance inputs for sustainable agricultural systems. Recent discoveries of copious production of glycoprotein (glomalin) by arbuscular mycorrhizal (AM) fungi and the apparent recalcitrance of this material in soils led to the comparison between concentration of glomalin and aggregate stability. Stability was measured on air-dried aggregates rewetted by capillary action and then subjected to wet sieving for 10 min. Thirty-seven samples from four geographic areas of the U.S. and one area of Scotland were tested. The monoclonal antibody used to discover glomalin on AM hyphae was employed to assess immunoreactive glomalin on aggregate surfaces by immunofluorescence and in extracts from aggregates by enzyme-linked immunosorbent assay (ELISA). Immunofluorescence was observed on at least some surfaces of aggregates from all soils examined, but was most evident on aggregates with high glomalin concentrations. Easily extractable glomalin (EEG) was solubilized by 20 m*M* citrate, pH 7.0 at 121 °C for 30 min, and total glomalin (TG) was solubilized with 50 m*M* citrate, pH 8.0 at 121 °C for 90 to 450 min. Some soils required up to seven sequential extractions to remove all of the glomalin. Aggregate stability was linearly correlated ($p < 0.001$) with all measures of glomalin (mg/g of aggregates) in these soils. The best predictor of aggregate stability (AS) was immunoreactive easily extractable glomalin (IREEG) according to the following relationship: $AS = 42.7 + 61.3 \times \log_{10} IREEG$ ($r^2 = 0.86$; $p < 0.001$, $n = 37$).

Introduction

Aggregate stability is a way to estimate the ability of a soil to maintain good water infiltration rates, good tilth and adequate aeration for plant growth (Kemper and Rosenau, 1986; Emerson et al., 1986). Soil aggregation is a complex process that begins with consolidation of soil particles into microaggregates (<0.25 mm) and progresses to formation of macroaggregates from these smaller units (Tisdall and Oades, 1982). Previous studies have indicated the involvement of AM fungi and fine roots in water stable aggregate formation (Miller and Jastrow, 1990) in loamy soils. However, Degens et al. (1994) were unable to relate the amount of hyphae and fine roots in aggregates to stabilization through an enmeshing mechanism in a sandy loam soil. Emerson et al. (1986) and Oades (1993) reviewed the evidence for biological involvement in aggregate stabilization. Baldock and Kay (1987) suggest that structural stability may be more strongly influenced by some pools of soil carbon than others, thereby making total carbon less sensitive than a more specific source in predicting soil stability. Gijsman and Thomas (1995) and Haynes and Swift (1990) found positive correlations between aggregate stability and hot-water extractable carbohydrate concentration. However, the sources of carbon compounds involved in aggregate stabilization are not known (Baldock et al., 1987; Haynes and Swift, 1990).

The current work is based upon two recent discoveries: (i) an immunoreactive glycoprotein, glomalin, is produced by hyphae of arbuscular mycorrhizal (AM) fungi (Wright et al., 1996) and (ii) this protein is abundant in soils (Wright and Upadhyaya, 1996). Glomalin

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is insoluble in water and extraction from hyphae or soil requires citrate at neutral to alkaline pH and 121 °C. The extreme conditions required for solubilization of glomalin prevented discovery of this molecule until immunoreactive material on AM hyphae was detected by an antibody (Wright et al., 1996). The necessity to identify the reactive material led to use of the harsh and stringent extraction mentioned above. Such unusual conditions for extraction suggested that this molecule is very stable. Glomalin, a glycoprotein with N-linked oligosaccharides (manuscript submitted), has some characteristics of hydrophobins which are small proteins produced by fungi (Wessels, 1993, 1996). Insolubility and difficulty in extraction are common to hydrophobins and glomalin. Also, formation of a hydrophobic scum, as has been described for a fungal hydrophobin (Russo et al., 1982), was a distinctive characteristic noted on the surface of water used to maintain hydration of colonized roots removed from sand cultures in a time-course study for production of glomalin on hyphae (Wright et al., 1996). This was the first indication of the large amount of glomalin produced during active colonization of roots by AM fungi. The abundant production, apparent recalcitrance, and hydrophobic characteristics indicated that glomalin might be involved in stabilization of aggregates.

Actively growing hyphae produced glomalin with consistently high immunoreactivity for all AM fungi tested to date (Wright et al., 1996 and unpublished data). Glomalin was extracted from hyphae by 20 m *M* citrate, pH 7.0 at 121 °C for 1 h (Wright et al., 1996), and modifications of this extraction method were tested on a number of soils (Wright and Upadhyaya, 1996). Glomalin from soil extracted with 20 m*M* citrate, pH 7.0 and 30 min at 121 °C [easily extractable glomalin (EEG)] has more of the immunoreactive fraction than has glomalin extracted by exposure to higher molarity citrate and longer heating cycles [total glomalin (TG)]. Different fractions of glomalin may exist in soil because of changes over time that affect the binding site of the antibody used to detect glomalin. Protein in crude extracts from soils has the same electrophoretic banding on sodium dodecyl sulfate gels and other general characteristics as had protein extracted from actively growing AM hyphae (Wright and Upadhyaya, 1996; unpublished data). Therefore, significant amounts of extraneous proteins probably are not being extracted by these methods, but apparently there is a fraction of soil glomalin that is immunoreactive. The current work extends the previous studies to examination of aggregates from soils with geographic and cropping diversity. The purpose of this survey was to determine whether current levels of glomalin in aggregates could be related to stability, and these measures would integrate temporal differences in AM fungal species and activity, plant species, other soil microorganisms, and anthropomorphic influences along with different soil textures.

Materials and methods

Soils

Soils used represented a variety of geographic regions, general characteristics, and cropping histories as shown in Table 1. All samples were taken from the A-horizon or the top 15 cm. Mid-Atlantic states soils from undisturbed woodlands were collected as batch samples from ca. 1-meter square areas. Two additional samples of the Beltsville soil were mixtures of three sub-samples taken from ca. 1 ha areas within plots. Illinois soils are mixtures of five sub-samples taken from 1.2 ha plots or equivalent areas of larger plots. One sample was from a fence-row between plots. Texas soils were sampled as ca. 1 kg taken from a site within a field. All of the above samples were received as air-dried bulk samples and were sieved to remove 1-2 mm aggregates. Minnesota soils are from 11 × 11 m plots in an area being studied for biodiversity effects on the establishment native prairie species of grasses and forbs. Composites of 2.5 x 15 cm cores were taken from six plots of 1 spp., six plots of 2 spp., and a nearby undisturbed area. Scottish samples were taken from near the vegetation identified in Table 1. Both Minnesota and Scottish samples were air dried, and 1–2 mm aggregates were sieved before samples were shipped.

Immunoreactive material (IM) on the surface of aggregates and AM fungal hyphae

Hyphae from a four-month-old pot culture of *Gigaspora gigantea* (Nicol. and Gerd.) Gerdemann and Trappe MA453A was examined. The solid phase used for attachment of hyphae during colonization of roots and for the subsequent immunoassay was a piece of polyethylene horticultural film. This porous film was placed in the bottom of a pot to retain sand and to allow for drainage of nutrients during colonization of Sudangrass (*Sorghum sudanense* (Piper) Staph) (Millner and Kitt, 1992).

Table 1. Selected information on soils used in this experiment

Geographic region Soil region	Soil	Cropping history[c]	% C[a,b]	pH[a] (1:1 H_2O)
Mid-Atlantic States	Beltsville series silt loam Fine-loamy, mixed, mesic Typic Fragiudults	Woodland, second growth, oak, sweetgum, maple, and pine	2.61	4.5
		Alleyway in an apple orchard	2.67	6.4
		Soybean–corn rotation	3.8	5.5
	Berks series channery loam Loamy-skeletal, mixed mesic Typic Dystrochrepts	Woodland, second growth oak, maple, and pine; under story mountain laurel, huckelberry	2.87	3.6
	Baltimore series silt loam Fine-loamy mixed, mesic Mollic Haplaudalfs	Woodland, second growth, oak, maple, and pine; under story: greenbrier, black gum	2.63	5.2
	Cullen series laom Clayey, mixed, mesic Typic Haplaudalts	Woodland, second growth, poplar, pine, and oak; under story: flowering dogwood, black haw, black gum	6.26	3.6
	Dekalb series channery sandy loam Loamy-skeletal, mixed, mesic Typic Dystrochrepts	Woodland, second growth, locust and oak; under story: blueberry, mountain laurel	11.09	4.0
	Cecil series sandy loam Clayey, kaolinitic, thermic Typic Hapludults	Woodland, second growth, poplar, oak, and hickory; under story: black gum, flowering dogwood, hickory,red maple	3.19	4.3
	Georgeville series silt loam Clayey, kaolinitic, thermic Typic Hapludults	Woodland, second growth, pine; under story: black gum, eastern red cedar	2.63	3.8
	Gilpin series channery silt loam Fine–loamy, mixed, mesic Typic Hapludults	Woodland, second growth, oak and hickory; under story: flowering dogwood, yellow poplar, greenbrier, Virginia creeper.	3.10	4.3
	Hagerstown series silt loam Fine, mixed, mesic Typic Hapludalfs	Woodland, second growth, cherry, maple, and oak; under story: greenbrier, flowering dogwood	4.56	6.6
	Manor series loam Coarse–loamy, micaceous, mesic Typic Dystrochrepts	Woodland, second growth, maple, oak, and pine; under story greenbrier	6.48	4.8
	Tatum series silt loam Clayey, mixed, thermic Typic Hapludults	Woodland, second growth, pine and oak; under story huckleberry, red maple, chestnut oak	2.55	3.9
Illinois	Sable series silty clay loam Fine-silty, mixed, mesic Typic Endoaquolls	Corn-soybean rotation	3.49	6.5
		Corn-soybean rotation	4.41	6.0
		Fence-row	4.06	6.6
		Corn-corn-hay-hayrotation	3.06	6.0
		Corn-soybean-wheat-vetch rotation	3.00	5.9
		Corn-soybean-wheat rotation	3.74	6.3

Table 1. continued

Texas	Berda series loam	Dryland cotton	0.57	8.4
	Fine-loamy, mixed, superactive, thermic Aridic Ustochrepts	Native grasses	0.85	8.2
	Posey series fine sandy loam	Abandoned farm field cultivated a year prior to sampling to control weeds	0.43	8.0
	Fine–loamy, mixed, thermic Calcíorthidic Paleustalfs	Grass pasture	0.93	7.7
		Dryland cotton	0.81	8.1
		Native grasses, not pastured	1.02	8.0
	Mansker series loam	Rangeland	0.90	8.1
	Fine–loamy, carbonatic, thermic Calciargidic Paleustolls	Rangeland	0.85	8.0
Minnesota	Nymore series loamy sand	The A-horizon was removed and the area was planted with one native species two years before sampling	1.58	5.5
	Mixed, frigid Typic Udipsamments	The A-horizon was removed and the area was planted with two native species two years before sampling	1.60	5.3
		The A-horizon was undisturbed and had been a bromegrass ca. 40 y	1.46	5.6
Dundee Scotland	Carey series of the Carpow Association	Hawthorn with broom	3.96	6.7
	Shallow sandy clay loam overlying red sandstone boulders; the soil was derived from rock quarry waste	Hawthorn	7.83	6.8
		Broom mosaic in grassland	4.29	6.4
	Grassland is in the early stage of secondary succession; woodland is hawthorn in late stages of secondary succession (elm predominated over hawthorn prior to removal of elm for phytosanitary reasons)	Grassland	3.08	5.8
		Woodland, in relatively open area	10.02	6.4
		Woodland, in shade	8.19	6.5
		Woodland, in deep shade	9.37	6.6

[a] For 1– 2 mm aggregates.
[b] Percent carbon was determined by combustion. All of the Texas soil samples were acidified to pH 5.8 prior to this analysis.
[c] Scientific names of plants mentioned in this table. Trees: apple (*Malus domestica* Borkh.), ash (*Fraxinus* spp.), black walnut (*Juglans nigra* L.), elm (*Ulmus* sp.), hawthorn (*Crataegus* spp.), hickory (*Carya* spp.), locust (Robinia spp.), maple (*Acer* spp.) oak (*Quercus* spp.), pine (*Pinus* spp.), poplar (*Populus* spp.), red maple *(Acer rubrum* L.), sweetgum (*Liquidambar styraciflуna* L.). Under story: black gum (*Nyssa sylvatica* Marsh.), black haw (*Vibrunum prunifolium* L.), blueberry (*Vaccinium* spp.), chestnut oak (*Querrus prinus* L.), eastern red cedar (*Juniperus virginiana* L.), flowering dogwood (*Cornus florida L.*), greenbrier (*Smilax rotindifolia L.*), huckleberry (*Rhododendron arboreum* Sm.) mountain laurel (*Kalmia latifolia* L.), Virginia creeper (*Parthenocissus quinquefolia* (L.) Planch), wild cherry (*Prunus ilicifolia* (Nutt. ex Hook. & Arnott) Walp.), yellow poplar (*Liriodendron tulipifera* L.). Crops and other plants: corn (*Zea mays* L.), cotton (*Glossypium hirsutum L.*), broom (*Cytisus scoparius* (L.) Link), bromegrass (*Bromus* sp.), soybean (*Glycine max* L.), vetch (*Vicia* sp.), wheat (*Triticum aestivum* L.).

Aggregates from samples described in Table 1, collected and processed as described above, were examined for surface immunofluorescent material. The immunofluorescence assay was performed as described for hyphae attached to roots (Wright et al., 1996). Briefly, 1-cm square pieces of horticultural film or ca. 20 aggregates in 1 cm diameter 52 μm mesh sieves were placed in a well of a 12-well tissue culture plate. Samples were reacted sequentially with blocking agent, monoclonal antibody MAb 32B11, and a fluorescein isothiocyanate (FITC)-tagged anti-mouse IgM (μ-chain reactive) secondary antibody with thorough washing to remove unattached antibodies. Aggregates or a piece of horticultural film were placed in a large drop of mounting medium on a microscope slide and covered with a cover glass. Immunofluorescence on the surface of aggregates was assessed. Controls were samples reacted with all reagents except the MAb, or an anti-*Rhizobium* MAb was substituted for MAb 32Bll.

Easily extractable (EEG) and total glomalin (TG) fractions in aggregates

Replicate 0.25 g samples of dry-sieved 1–2 mm aggregates were extracted with 2 ml of extractant. EEG was extracted with 20 m*M* citrate, pH 7.0 at 121 °C for 30 min. TG was extracted with 50 m*M* citrate, pH 8.0 at 121 °C. The time required was 90 min for all samples except those from Illinois and Scotland which required up to six additional sequential extractions. For sequential extractions, the supernatant was removed by centrifugation at 10,000 $\times$ *g* for 5 min, 2 mL of 50 mm citrate, pH 8.0 was added to the residue, and samples were autoclaved for 60 min. Extraction of a sample continued until the supernatant showed none of the red-brown color typical of glomalin. Extracts from each replicate were pooled and then analyzed.

Citrate extractants were added to calcareous soil samples and the aggregates were disrupted by a brief (3 min) autoclave cycle. When necessary, the extractant was adjusted with HCl until the pH stabilized at 7.0 for 20 m*M* citrate or 8.0 for 50 m*M* citrate. Samples were then subjected to 121 °C for 90 min to extract TG or 30 min to extract EEG.

After extraction cycles were completed, samples were centrifuged to remove the soil particles (10,000 $\times$ *g* for 5 min), and protein in the supernatant was determined by the Bradford dye-binding assay with bovine serum albumin as the standard (Wright et al., 1996). Concentration of glomalin was extrapolated to mg/g of aggregated soil particles by correcting for the dry weight of coarse fragments $>$0.25 mm included in the weight of aggregates and for the volume of extractant.

Enzyme-linked immunosorbent assay (ELISA) of extracts

The ELISA value of 0.125 μg protein extracted from aggregates was determined by the procedure described by Wright et al. (1996) except that the secondary antibody was biotinylated anti-mouse IgM (μ-chain reactive) antibody with biotin attached via a long spacer arm (Jackson ImmunoResearch Laboratories, Inc., Westgrove, PA). The long spacer arm biotinylated antibody apparently overcame a problem with stearic hindrance caused by the multiple layers of antibodies at the reactive site and resulted in a four-fold increase in sensitivity over the previously reported procedure (Wright and Upadhyaya, 1996). ELISA value (A_{405}) of 0.125 μg protein from extracts of aggregates was compared with that of the same amount of glomalin extracted (20 m*M* citrate, pH 7.0, 121 °C for 1 h) from fresh AM fungal hyphae. Immunoreactive easily extractable glomalin (IREEG) and immunoreactive total glomalin (IRTG) fractions were determined.

Aggregate stability

All soils had been stored as air-dried samples for $>$4 months. Replicate 4 g samples of 1–2 mm aggregates were moistened with deionized water by capillary action for 10 min. Aggregates were subjected to wet sieving for 10 min in the apparatus described by Kemper and Rosenau (1986). Soil particles passing through a 0.25 mm sieve were dried at 105 °C and weighed. Material remaining on the 0.25 mm sieve was dispersed with 5% sodium hexametaphosphate and the coarse material was washed with deionized water, dried at 105 °C and weighed. The initial and final weights of aggregates were corrected for the weight of coarse particles ($>$0.25 mm). Aggregate stability is the mass of aggregated soil remaining after wet sieving as a percent of the total mass of soil.

Statistical analysis

Statistix (Analytical Software, Tallahassee, FL) software was used to calculate Pearson correlation coefficients and linear regression models. For this survey each sample was considered separately to test the relationship between glomalin and aggregate stability. Aggregates were from a diversity of soils with the

influence of a diversity of plants and degrees of disturbance.

Results

Photomicrographs of immunoreactive material (IM) on aggregates from Cecil soil are shown in Figure 1. The reaction was typical of FITC immunofluorescence (i.e. bright apple green) and is shown in Figure 1A as white areas. IM is highly concentrated on the aggregate in some areas. In Figure 1B hyphae (H) are attached to an aggregate and IM on the surface of hyphae can be seen. Diameter of these hyphae indicate that these are within the size range of AM fungal hyphae (2-8 μm). Immunoreactive material on hyphae of *Gigaspora gigantea* MA453A is shown in Figure 1C for comparison. Many small circular pieces of IM, apparently sloughed from hyphae, also are present. Horticultural film pieces that had not been used in pot cultures or were tested with reagent controls showed no fluorescence.

There was variation in the amount of IM on aggregates from different soils, but, in general, at least some of the surface of aggregates from all samples tested had IM. There was an observed correlation between high concentration of extracted immunoreactive glomalin and the amount of immunoreactive material on the surface of aggregates. Controls showed no immunofluorescence and autofluorescent material was easily distinguished from IM because of color (yellow) or texture. Some individual particles within aggregates, identified by size and surface smoothness as sand, had a green autofluorescence in controls. The smooth appearance of the entire fluorescent particle readily distinguished autofluorescence from the rough patchiness characteristic of IM associated with glomalin.

Table 2 shows correlation coefficients for fractions of glomalin, percent carbon in aggregates, and aggregate stability. All fractions of glomalin were highly correlated with each other and with aggregate stability of soil samples. Percent carbon also was highly correlated with aggregate stability and with each of the four fractions of glomalin.

Means and standard deviations for aggregate stability and measures of glomalin in soils from various regions are shown in Figure 2. Concentrations of TG ranged from 1 to 21 mg/g for a calcareous Texas soil in dryland cotton and a Scottish woodland sample, respectively.

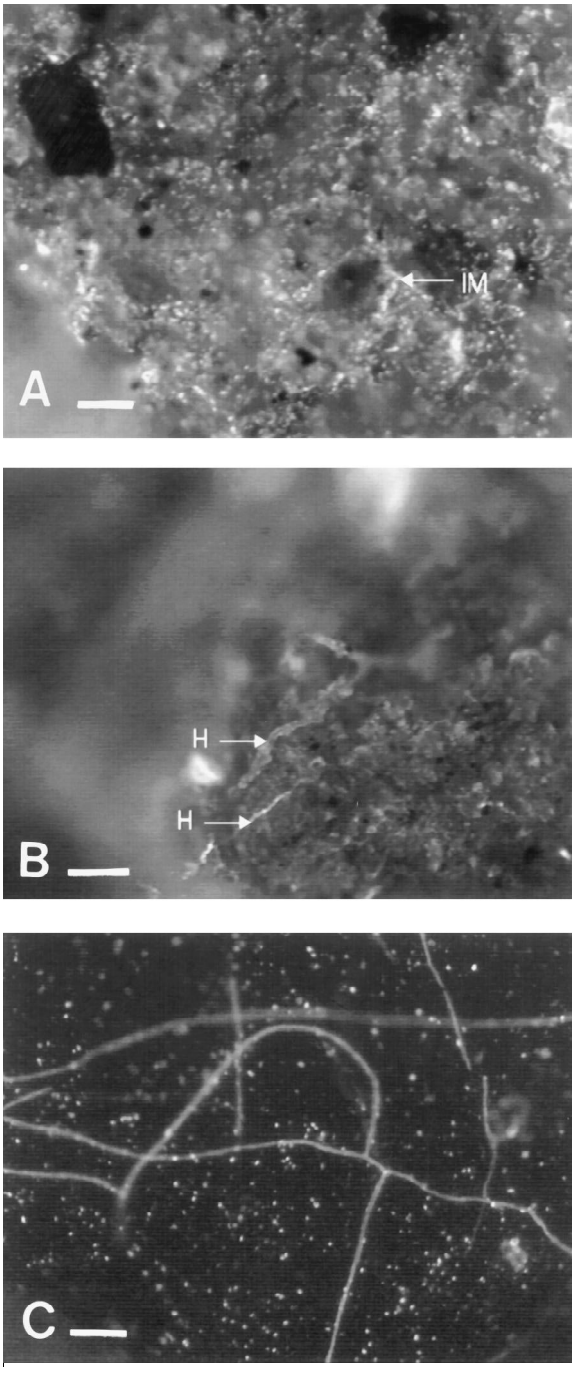

Figure 1. Glomalin, shown as immunoreactive material (IM) on soil aggregates and hyphae of an arbuscular mycorrhizal fungus. (A) an aggregate from a mid-Atlantic states soil (Cecil soil series) collected from an undisturbed area shows IM on the surface. (B) shows IM on hyphae (H) on an aggregate from the same soil. (C) shows IM on hyphae of an isolate of *Gigaspora gigantea* attached to a piece of plastic horticultural film. Numerous small pieces of IM were seen as small dots on the horticultural film. (Bar = 60 μm)

Table 2. Correlation coefficients for measures of glomalin, total carbon, and aggregate stability for a survey of 37 soil samples representing a variety of soil types and differences in cropping histories within some of the soils

	%C	Easily extractable[a]	Immunoreactive[b] easily extractable	Total[c]	Immunoreactive total
Easily extractable	0.49**				
Immunoreactive easily extractable	0.61***	0.94***			
Total	0.82***	0.73***	0.78***		
Immunoreactive total	0.79***	0.60***	0.74***	0.90***	
Aggregate stability (%)[d]	0.65***	0.69***	0.84***	0.70***	0.79***

[a] Protein concentration in aggregates (0.25 g) extracted with 2 ml 20 m*M* citrate, pH 7.0 at 121 °C for 30 min. The Bradford dye-binding assay value of extracts, with bovine serum albumin as the standard, was extrapolated to mg/g aggregates.
[b] Enzyme-linked immunosorbent assay (ELISA) value (A_{405}) for 0.125 μg of protein compared with the ELISA value of the same amount of glomalin from *Gigaspora gigantea* MA453A was used to calculate the concentration of immunoreactive glomalin in extracts. This value was extrapolated to mg immunoreactive glomalin/g aggregates.
[c]Protein in aggregates extracted with 2 ml 50 *M* citrate, pH 8.0 at 121 °C for 90 min except for Illinois and Scottish samples which were extracted an additional 6 h. Extractant was removed and 2 ml fresh extractant was added for each addition 1-h cycle.
[d] Aggregate stability was tested after rehydration of air-dried samples by capillary action for 10 min. The values is the mass of aggregated soil remaining as a percent of the total mass of soil after 10 min of wet sieving. **,**** denote significance at less than 0.1 and 1% levels, respectively.

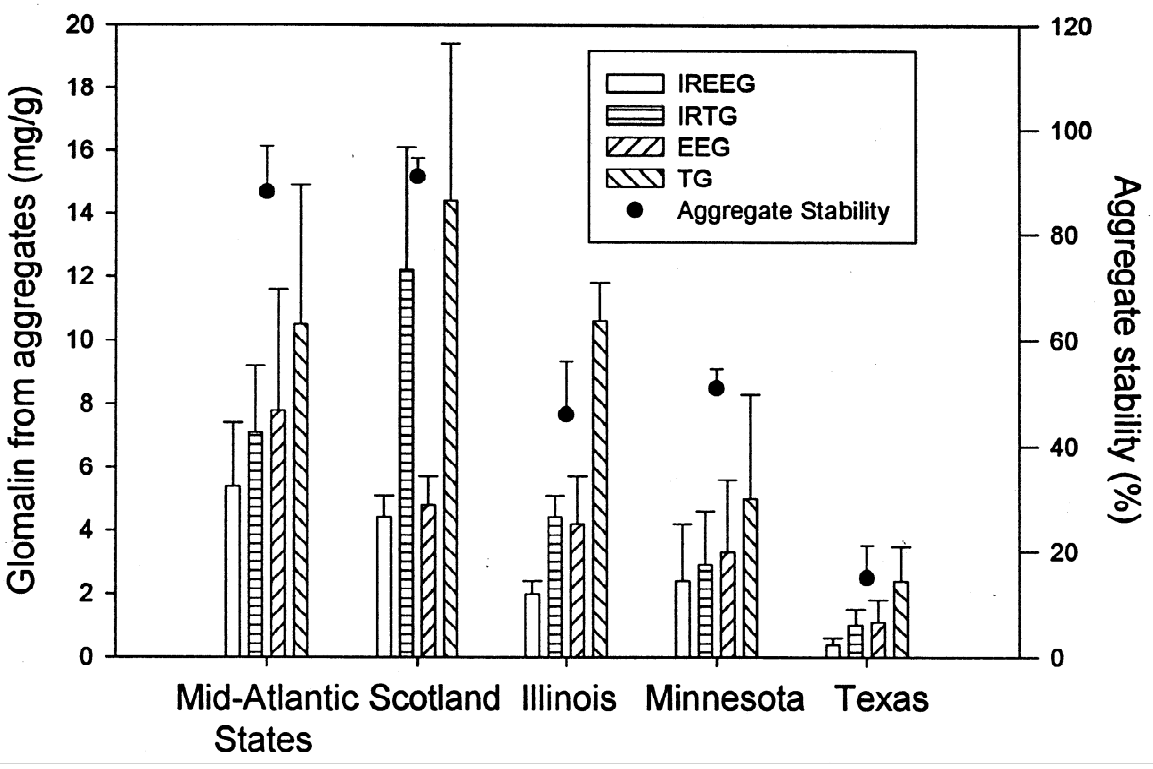

Figure 2. Means and standard deviations of fractions of glomalin and aggregate stability in soils from four areas of the USA and one area of Scotland. IREEG = immunoreactive easily extractable glomalin, EEG = easily extractable glomalin, IRTG = immunoreactive total glomalin, and TG = total glomalin.

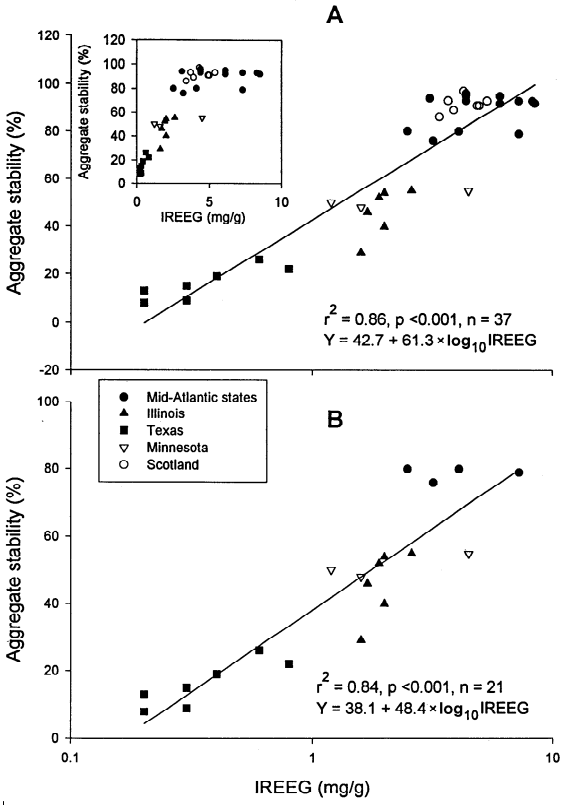

Figure 3. Relationship between stability of 1–2 mm-size aggregates and immunoreactive easily extractable glomalin (IREEG) soils from four areas in the USA and one area in Scotland is shown for $\log_{10}$ (A) and linear scales (insert) of IREEG. The relationship between samples with $\leq$80% aggregate stability and IREEG is shown in (B).

Figure 3A shows a highly significant relationships between aggregate stability and $\log_{10}$IREEG for samples that had wide ranges in these values. The inset in Figure 3A is a plot of these data on the linear scale for IREEG to show the bimodal nature of the relationship between aggregate stability and IREEG. After 80% aggregate stability, an apparent hyper-accumulation of glomalin occurred in some samples.

Other fractions of glomalin also showed significant relationships ($p < 0.001$) with aggregate stability (AS) as described by the following equations: AS = 5.7 + 68.0$\times\log_{10}$TG (r^2 = 0.58); AS = 29.6 + 63.4$\times\log_{10}$EEG (r^2 = 0.67); and AS = 22.6 + 66.1$\times\log_{10}$IRTG (r^2 = 0.81). These other fractions of glomalin showed the same relationships among geographic groups of soils as illustrated for IREEG in Figure 3A.

Soils from the mid-Atlantic states and Scotland had high concentrations of glomalin and were the most stable. Sixteen samples from the mid-Atlantic states and Scotland had aggregate stabilities >80%. Values for IREEG in the remaining 21 samples were used to test whether the relationship between IREEG and aggregate stability was skewed by high values that possibly represented hyper-accumulation of glomalin. Figure 3B shows that the relationship between IREEG and aggregate stability was maintained for samples with aggregate stability $\leq$80%.

Values for IREEG were highly correlated with values for EEG (Table 2). Linear regression of these measures of glomalin had an r^2 = 0.89 for IREEG = 0.27 + 0.64$\times$EEG (n = 37). Samples with aggregate stabilities of $\leq$80% showed a linear relationship between EEG and IREEG: IREEG = $-$0.14 + 0.65$\times$EEG (r^2 = 0.94, n = 21). Thus, EEG could be used to evaluate aggregate stability if the monoclonal antibody against glomalin were not available.

Disturbed and undisturbed samples from Texas, Minnesota, and the Beltsville mid-Atlantic states soil (Table 1) were compared (Table 3). Texas samples had very weak aggregate stability (<10% for disturbed sites and <30% for undisturbed sites). IREEG was greater for undisturbed compared with disturbed sites. Minnesota samples were composites of two disturbed and one undisturbed site. Values for IREEG indicated a marked effect of the removal of the A-horizon 2y prior to sampling. Beltsville soil represented samples for comparison between undisturbed and disturbed sites within the wide range of aggregate stability measured in this study. Differences in aggregate stabilities among the samples were seen, but IREEG values were very close for samples with 80 and 93% aggregate stability, representing disturbed and undisturbed sites, respectively.

Discussion

These results indicate that glomalin is highly correlated with aggregate stability, measured as resistance to breakdown by wet sieving of air-dried samples, for a variety of soils. Easily extractable glomalin and the immunoreactive fractions of glomalin in aggregates were more closely correlated with aggregate stability than TG. The immunoreactive site is not affected by up to an additional 120 min of autoclaving after extraction (data not shown). Therefore, the generally lower amount of immunoreactive glomalin in TG compared with EEG was because a larger amount of non-

Table 3. Comparison of immunoreactive easily extracted glomalin (IREEG) and aggregate stability values for disturbed and undisturbed samples from three different soils.

Soil	Disturbed[a]		Undisturbed	
	IREEG[b] (mg/g)	Aggregate stability (%)[c]	IREEG (mg/g)	Aggregate stability (%)
Posey fine sandy loam	0.3	9	0.8	22
(Texas)	0.2	8	0.6	26
Nymore loamy sand	1.2	50	4.5	55
(Minnesota)	1.6	46		
Beltsville silt loam	3.2	76	4.4	93
(Mid–Atlantic States)	4.1	80		

[a] Soils had been subjected to various degrees of disturbance by tillage or removal of the top 15 cm within two years of sampling.
[b] Protein concentration in aggregates (0.25 g) extracted with 2 ml 20 m*M* citrate, pH 7.0 at 121 °C for 30 min was measured by the Bradford dye-binding assay value of extracts, with bovine serum albumin as the standard. Enzyme–linked immunosorbent assay (ELISA) value (A_{405}) for 0.125 μg of protein compared with the ELISA value of the same amount of glomalin from *Gigaspora gigantea* MA453A was used to calculate the concentration of immunoreactive glomalin in extracts. This value was extrapolated to mg immunoreactive glomalin/g aggregates.
[c] The value is the mass of aggregated soil remaining as a percent of the total mass of soil after 10 min of wet sieving.

immunoreactive glomalin was extracted by the longer heating cycle for TG.

Total glomalin in some clays may be very abundant, but it is tightly bound as indicated by the number of sequential extractions that were required for Illinois and Scottish samples. Also, the TG fraction in some samples may have undergone a conformational change that resulted in loss of reactivity with the antibody used in this study. Total glomalin may represent at least some portion of extremely persistent organic matter in soil (Tisdall and Oades, 1982).

The most stable aggregates had higher EEG and IREEG values than the less stable aggregates. If EEG and IREEG represent recent deposits or deposition protected from degradation, the continued production of glomalin by AM hyphal networks or minimizing the exposure of protected glomalin to degradation may be essential to maintain soil stability. Alternatively, the effects of soil disturbance on aggregates can last for many years (Jastrow, 1987; Low, 1955), and this may be explained by disruption of hyphal deposition and possibly enhanced destruction or modification of glomalin. The strong linear relationship between EEG and IREEG for samples with $\leq$80% aggregate stability (r^2 = 0.95) indicates that EEG may be a useful and simple measurement to evaluate relationships between aggregate stability and agronomic practices.

Soils used in this study were from different geographic locations and had different textural characteristics. Disturbed and undisturbed samples of some soils in the current study indicated that further work on the relationship between glomalin and aggregate stability for a single soil should be conducted. Spatial variability within a small sample area also needs to be evaluated.

Analysis of glomalin purified from the crude citrate extract by precipitation with ammonium sulfate and then dialyzed extensively against deionized water indicates that iron is associated with the molecule. Iron probably is the chromophore imparting the red-brown color to extracts. Glomalin from the 11 bulk samples of mid-Atlantic states soils used in this study had 0.8–8.8% Fe as determined by atomic absorption spectroscopy (unpublished data). It has been speculated that materials associated with polyvalent metal cations and polymers are involved in persistence of aggregates (Emerson et al., 1986; Tisdall and Oades, 1982). Iron deficiency, as occurs in calcareous soils, may be the reason for low glomalin values and, therefore, poor aggregate stability in Texas calcareous soils. Kemper and Koch (1966) found that aggregate stability was a function of organic matter, clay and free iron oxides for soils from the Western U.S. and Canada. Further work is in progress to determine the influence of available

iron on production of glomalin by selected AM fungal isolates.

Hot water extraction of carbohydrates (Gijsman and Thomas, 1995) shows a relationship between aggregate stability and carbohydrate concentration with a plateau at about 78% stability after which a further increase in carbohydrate concentration does not correlate with increasing aggregate stability. This relationship also apparently occurs with IREEG and aggregate stability as shown in the inset of Figure 3A. Hot water extractable carbohydrates measured by Gijsman and Thomas (1995) on a single soil were 0.4–1.2 mg/g. The largest total amount of extractable carbohydrates from a long-term pasture soil with stable aggregates, was 2 mg/g (Haynes and Swift, 1990). Concentrations of TG measured in the current work on samples with high aggregate stabilities (>80%) ranged from 4.8 to 21 mg/g. Texas soils had TG concentrations within the range of hot water extractable carbohydrates reported by Gijsman and Thomas (1995) and Haynes and Swift (1990), but also had the weakest aggregation of the samples tested. Previous work showed that glomalin is not extracted by water at 121 °C (Wright and Upadhyaya, 1996). Therefore, glomalin, a glycoprotein made up of approximately 60% carbohydrate (unpublished data), may be present in aggregates in amounts from 3 to 10 times greater than hot-water extractable carbohydrates. The relationship between of concentration of hot-water extractable carbohydrates, concentration of glomalin, and aggregate stability needs further study.

Aggregates used in this study had been stored in the air-dried state for greater than 4 months. Air-drying and storage can cause aggregate stability to increase according to previous reports (Haynes and Swift, 1990; Kemper and Rosenau, 1984; Utomo and Dexter, 1981). Slow rewetting of air-dried aggregates before wet sieving is a way to overcome the effects of air-drying. Soils used in the current study also were tested after slow rewetting with water droplets produced by a humidifier (Kemper and Rosenau, 1986). Results (data not shown) indicated that this rewetting method increased aggregate stability by 2–60% over more rapid rewetting by capillary action. Samples from mid-Atlantic states showed smaller increases than other soils, and Texas samples showed the greatest increases. Overall, aggregate stability of droplet rewetted samples showed the same general relationships among samples and for the geographic groups of samples as shown in Figures 2 and 3. Aggregate stability of air-dried samples rewetted by capillary action rather than by fine drops of water before testing was used to emphasize differences across a variety of soils and differences within a soil that may be due to cropping history. Rationale for using aggregate stability of air-dried samples, as opposed to field-moist samples in studies on organic binding agents is discussed by Haynes and Swift (1990).

Other measures of soil aggregate stability need to be tested for their relationship with glomalin. Haynes and Swift (1990) used mean weight diameter of aggregates in their study of aggregate carbohydrates. Measures of force required for aggregate disintegration by impact and abrasion also should be tested (Kemper and Rosenau, 1986).

According to the results presented here, small increases in IREEG in aggregates may lead to significant increases in stability. This type of soil stability would lead to greater sustainability of agricultural systems.

We speculate that AM fungi produce copious amounts of glomalin to create an environment for growth of their hosts, almost all vascular plants. These fungi are obligate phytotrophs and are ensuring their survival by increasing the probability that host plants will be able to grow at the site of fungal spores and reproductive hyphae by creating a hospitable soil environment. It is possible that glomalin simply contributes to hydrophobicity of soil particles to allow for air penetration and water drainage. It is also possible that the insoluble, glue-like, hydrophobic nature of a glomalin coating may initiate and protect nascent aggregating material – soil minerals, other microbes, and organic matter. Insoluble glomalin in its native state could trap and protect the microbes that contribute polysaccharides and other by-products to aggregates. A stable, well-aggregated soil structure maintains aeration and moisture conditions essential for optimal growth of plants (Oades, 1984).

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